Abstract: P314

Osteopontin stimulates cell cycle in neonatal cardiomyocytes

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Introduction: Macrophages control myocardial regeneration in the neonatal heart and repair in the adult heart. Osteopontin (OPN) is a macrophage-derived protein which is upregulated following tissue injury. While OPN regulates cell adhesion, spreading and migration, its role in myocardial regeneration is unknown.

Purpose: We aimed to test the hypothesis that OPN stimulates cardiomyocyte proliferation.

Methods and results: Mouse neonatal cardiomyocytes were treated with a serum-free medium for 24 hours. Immunofluorescent staining showed that these cells expressed the OPN receptor CD44 (Fig.1A). To assess cell expansion, we used MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, colorimetric assay. OPN preserved cardiomyocyte count in a low glucose medium for 24 hours, compared with no OPN treatment (Fig.1B). Staining for phosphohistone (pH)3, a marker of nucleus division, showed a higher expression of pH3 in cardiomyocytes after OPN treatment (Fig.1C). Immunoblotting revealed that OPN induced the phosphorylation of cell-cycle activity proteins, known as yes-associated protein (YAP)1, and extracellular signal-regulated kinase (ERK)2, while it decreased the phosphorylation of the YAP1 inhibitor, the large tumor-suppressor kinase (LATS)1/2 (Figs.1D and 1E). Gene expression analysis revealed that OPN upregulated the transcriptional enhancer factor TEF-1 (TEAD1), which interacts with YAP1 (Fig.1F), connective tissue growth factor (CTGF) and cyclin-dependent kinase 1 (CDK1), which are YAP downstream targets (Figs.1G and 1H). Furthermore, OPN significantly upregulated cyclin B1, a regulator of the mitotic (M) phase (Fig.1I). Moreover, OPN may promote positive feedback by upregulating the expression of CD44, SPP-1 (Figs.1J and 1K). Finally, administration of OPN to adult mouse improved cardiac remodeling and function after myocardial infarction.

Conclusions: OPN stimulates cell-cycle activity in neonatal cardiomyocytes through YAP1 activity, independently from the HIPPO-YAP signaling pathway. Because macrophages are a major source of OPN after injury, our findings could explain the essential role of macrophages in neonatal heart regeneration and adult heart repair.
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